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Research

Laboratory Investigation of Cauliflower–Fungus–Insect Interactions for Cabbage Maggot Control

Jaka Razinger,[1,4] Matthias Lutz,[2] Jürg Grunder,[3] and Gregor Urek[1]

[1]Plant Protection Department, Agricultural Institute of Slovenia, Hacquetova ulica 17, Ljubljana, SI-1000, Slovenia, [2]Agroscope, Competence Division Plants and Plant Products, Schloss 1, Wädenswil, CH-8820, Switzerland, [3]School of Life Sciences and Facility Management, Zurich University of Applied Sciences, Campus Grueental, Wädenswil, CH-8820, Switzerland, and [4]Corresponding author, e-mail: jaka.razinger@kis.si

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Abstract

The cabbage maggot (also known as cabbage root fly [CRF]; *Delia radicum* L.) is a serious pest of brassicas. The pest's soil-dwelling larvae are especially damaging to young brassica transplants. In light of toxic soil insecticide phase-out novel biocontrol management solutions are sought for. Our research is focused on the development of a biological control strategy involving cauliflower plantlet inoculation with insect pathogenic fungi. This article presents the results of a laboratory investigation of cauliflower × microbe × CRF interactions. Seven isolates of fungi (entomopathogenic and rhizosphere-competent fungi and soil saprotrophs) were tested for their pathogenicity to CRF and their effects on cauliflower plantlets. The laboratory experiments were performed in sterilized substrate. Several strains significantly increased CRF mortality, some at par with a commercial bioinsecticide based on *B. bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae). All strains colonized the rhizoplane, however to varying extent. Some isolates were also reisolated from within healthy plant tissues and thus identified as endophytes. The method of applying conidia had a significant effect on survival and weight of seedlings and rhizoplane and endophytic colonization rates. Two *Metarhizium brunneum* Petsch (Hypocreales: Clavicipitaceae) isolates exhibited plant growth promotion effects when ungerminated seeds were coated with conidia. The ecological implications of plant × microbe × pest interactions and options for improving the effectiveness of a fungal-based biological CRF management strategy are discussed.

Key words: biological control, entomopathogenic fungi, plant–microbe–insect interaction, rhizosphere competence, soil pest

The cabbage maggot (*Delia radicum* L., Diptera: Anthomyiidae) or the cabbage root fly (CRF) is a significant pest of cabbage crops. Problems are caused after females lay their eggs within 5 cm of the stem base of the host plants (Mukerji 1971). The newly hatched larvae crawl through the soil to find and feed on roots or bore directly into the young stems and this can cause serious economic damage. Most challenging are the early fly generations as they affect early developing, high value crops (Bligaard 1999). Depending on the measures taken and the CRF population pressure, yield losses of up to a 100% are possible (Ferry et al. 2009). Especially problematic are direct damages when parts of plants for consumption (e.g., roots of radish, swedes, or turnips) are infested. Indirect damage caused by larvae feeding on nonconsumed tissue can be tolerated to a certain degree (Finch 1993, Herbst et al. 2017, Razinger et al. 2017). Often only the young transplants need chemical protection from CRF in commercial broccoli or cauliflower production (Bligaard 1999).

The range of organophosphate and carbamate insecticides, used to manage CRF in the past (Chandler and Davidson 2005), is declining because of environmental concerns. Accordingly, the (European Parliament 2009) promoted implementation of low-risk methods including nettings and fleeces, lime nitrogen, straw mulch, parasitoid/predator release, chemical cues, variety selection, intercropping, and altered planting time and densities (Straub 1988; Städler and Schöni 1990; Dosdall et al. 1996, 2000; Nawrocka 1996; Ferry et al. 2009; Hummel et al. 2010; Reddy 2011; Cotes et al. 2015; Joseph and Zarate 2015; Herbst et al. 2017). In addition, several articles report the possibility to manage CRF using entomopathogenic or insect-associated fungi (entomopathogenic fungi [EPF]) which can infect and kill dipterous insects (Vänninen et al. 1999; Klingen et al. 2002; Bruck et al. 2005; Thomas and Read 2007; Toledo et al. 2007; Razinger et al. 2014a,b; Cotes et al. 2015; Myrand et al. 2015; Rännbäck et al. 2015). Pest control through EPF could be optimized by selecting fungal species that well-align to the CRF lifecycle or associated cabbage crops as root colonizers and endophytes or soil saprotrophs. There is thus an increasing need to understand interactions between fungi, plants, and pests to increase the efficiency of pest control strategies with EPF.

This article presents the results of our ongoing research on evaluating EPF and potentially plant growth promoting fungi as CRF biocontrol agents. Selected fungal species were previously tested in glasshouse settings and in commercial field cauliflower production settings (Razinger et al. 2014b, Razinger et al. 2017). The present study focused on plant × microbe interactions under controlled laboratory conditions.

Materials and Methods

Fungal Pathogenicity to CRF

CRF eggs used in the in vitro pathogenicity bioassays were obtained from a laboratory CRF culture as described elsewhere (Razinger et al. 2014b). The fungal strains used were obtained and conidial suspensions prepared as described elsewhere (Razinger et al. 2014a). The tested fungi were *Metarhizium brunneum* (strains H.J.S. 1154 and 1868), *Beauveria bassiana* (Bals.-Criv.) Vuill. (H.J.S. 1174), *Clonostachys solani* f. *nigrovirens* (J.F.H. Beyma) Schroers (H.J.S. 1828) (Hypocreales: Bionectriaceae), *Trichoderma atroviride* P. Karst. (H.J.S. 1873), *T. koningiopsis* Samuels, C. Suárez & H.C. Evans (H.J.S. 1874), and *T. gamsii* Samuels & Druzhin. (H.J.S. 1876) (Hypocreales: Hypocreaceae). The ability of the fungi to infect CRF eggs or larvae and negatively affect their survival was tested. The test container was a 100 ml polypropylene plastic pot, into which a 9-cm sterile filter paper, moistened with 1.5 ml of sterile demineralized water, and a 5 × 20 × 20 mm piece of surface-sterilized rutabaga was placed. Five fresh CRF eggs, obtained from a laboratory CRF culture, were placed into the test container 35 mm from the rutabaga slices. A volume of 50 µl of 1×10^8 viable conidia ml^{-1} was pipetted onto the CRF eggs. The test containers were sealed with parafilm and put into an environmental chamber at 22°C, 77% relative humidity (RH), without illumination. Insecticide Marshal 25 CS (a.i. carbosulfan 24.5%; 0.1% (v/v) dilution; Maag Agro, Switzerland) was used as a positive control. Bioinsecticide Naturalis (a.i. *Beauveria bassiana* ATCC 74040; 0.1% (v/v) dilution; Andermatt biocontrol AG, Switzerland,) and bioinsecticide Delfin (a.i. *Bacillus thuringiensis* Beliner var. *kurstaki* (Bacillales: Bacillaceae); 0.5% (v/v) dilution; Andermatt biocontrol AG, Switzerland) were used as reference biocontrol agents. Tween 80 was used in the negative control treatment (0.05% (v/v) dilution), as this surfactant was used to prepare the conidial suspensions in the fungal treatments. Five separate test containers were made for each treatment and the experiment replicated three times independently ($n = 15$).

Cauliflower–Fungus Interactions in Multiwell Plates

Experimental set-up

The multiwell plate experiment was performed under controlled conditions at 77% RH, 18: 6 h at 20: 18°C day: night regime in sterile six-well plates. To each chamber of the six-well plate, 2.5 g of twice autoclaved sterile commercial planting substrate and one surface-sterilized seed of cauliflower cultivar 'Neckerperle' was placed. The seed surface sterilization was performed by immersing seeds for 3 min in 70% ethanol with three intervals of 10 s vortexing. Then, the seeds were rinsed with sterile demineralized water and allowed to dry in a laminar flow chamber. The surface sterilization procedure was tested by placing five surface-sterilized seed onto potato dextrose agar plates. No fungal colonies were observed after 1 wk incubation at 25°C.

Treatments

Fungi were inoculated into the test containers by placing sporulating agar plugs (malt extract agar for *Trichoderma* spp. and potato dextrose agar for other strains; diameter of 8 mm) below germinating seeds, or coating surface-sterilized seeds with conidia. For seed coating exposure, conidial suspensions of a concentration of 5×10^7 conidia ml^{-1} were prepared in 1% carboxymethyl cellulose (CMC; Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Eighty seeds were vortexed in 50 ml centrifuge tubes containing 10 ml of CMC-conidial suspensions for 30 s, then the excess conidial suspension was removed and the seeds spilled onto a sterile petri dish and allowed to dry in a laminar flow chamber. The amount of conidia attached to the seeds was estimated by washing the conidia off five seeds per fungal treatment in 0.05% Tween 80. The number of conidia was assessed by plating serial dilutions on 1.5% malt extract agar (Sigma–Aldrich Chemie GmbH). Washing and plating was performed in. The results of the coating procedure can be seen in Table 1. The 'plugs' treatment was performed in three wells per fungal strain and the experiment repeated independently twice ($n = 6$). The 'seed coating' treatment was performed in six wells per fungal strain and the experiment repeated independently three times ($n = 18$). The negative control comprised of surface-sterilized seeds in the 'plugs' treatment or surface-sterilized seeds coated with 1% CMC in the 'seed coating' treatment.

Evaluation

After 28 d, plantlet survival was calculated as the quotient between living plantlets and the number of input seeds. Additionally, plants' rhizoplane and endophytic colonization was evaluated as described elsewhere (Razinger et al. 2014b). In brief, five 1-cm-long root pieces were sampled per well to evaluate rhizoplane colonization. The root pieces were washed twice with tap water and five times with sterile demineralized water. The washed root pieces were transferred onto Strasser agar plates (Strasser et al. 1997) for the detection of *M. brunneum* (1154 and 1868), *B. bassiana* 1174 and *C. solani* 1828. *Trichoderma* spp. (1873, 1874, and 1876) treated plant material was transferred onto *T. harzianum*-selective medium (THSM; Williams et al. 2003). The Strasser plates were incubated for 14 d, and THSM plates for 4 d at 22 ± 2°C. Another collection of five washed root pieces, three stem pieces (one cm in length), or two leaves per well were surface sterilized for evaluating endophytic colonization. Surface sterilization was performed in 25-ml Falcon tubes in 10 ml 70% ethanol for 3 min. During the 3 min submersion, the tubes were vigorously vortexed three times for 10 s. The pieces were then washed with sterile demineralized water. No fungal colonies were encountered when 100 µl final wash-water was pipetted onto Strasser or THSM plates and incubated for 1 wk at 25°C.

Pot Experiment

Experimental set-up

The pot experiment was performed under controlled conditions at 77% RH, 18: 6 h at 20: 18°C day: night regime in 0.5-liter plastic

Table 1. The number of viable conidia per seed obtained during the coating procedure (means ± SE)

Treatment	Number of viable conidia per seed
M. brunneum 1154	$4.5 \times 10^3 \pm 2.3 \times 10^3$
M. brunneum 1868	$3.5 \times 10^3 \pm 1.0 \times 10^3$
B. bassiana 1174	$2.7 \times 10^4 \pm 5.8 \times 10^3$
C. solani 1828	$2.0 \times 10^4 \pm 5.4 \times 10^3$
T. atroviride 1873	$1.3 \times 10^4 \pm 3.5 \times 10^3$
T. koningiopsis 1874	$6.8 \times 10^3 \pm 6.9 \times 10^2$
T. gamsii 1876	$4.0 \times 10^3 \pm 1.2 \times 10^3$
Control	0.0 ± 0.0

pots. To each pot, 0.4 liters of twice autoclaved sterile commercial planting substrate were placed. Eight surface-sterilized seeds of cauliflower cultivar 'Neckerperle' were placed into each pot. Three pots per treatment were considered replicates; the experiment was repeated twice independently (n = 6). The seed surface sterilization was performed as in the multiwell plate experiment described above.

Treatments

The fungi were applied by inoculating the growing substratum via drenching, or by adding conidia-coated seeds, prepared as described in the multiwell plate experiment. The pots were watered with 30 ml of autoclaved demineralized water. In the drenching exposure, 15 ml of water was replaced with conidial suspensions of a concentration of 5.3×10^5 viable conidia ml^{-1}. Three separate negative controls were performed. In 'Zero control', untreated seeds were used. In the drenching application, negative control seeds were surface sterilized but drenched only with sterile demineralized water. In the seed coating application, negative control seeds were surface sterilized and coated with 1% CMC, without conidia.

Evaluation

After 28 d, plantlet survival was evaluated. The plantlets were carefully dug out with the help of tweezers and a spatula. The roots were washed with tap water to remove any adhering test substrate, and blotted on a paper towel to remove any excess tap water. Blotted plantlets were weighed using a high precision laboratory scale (BP 301 S, Sartorius, Germany).

Data Analysis

Results from fungal pathogenicity tests were corrected using Abbott's formula that eliminates errors due to deaths in the control sample (Abbott 1925), and their normality of distribution tested by D'Agostino–Pearson omnibus K2 test. Abbott's mortalities were tested by analysis of variance (ANOVA) and Student's t-tests. The proportion of surviving plantlets and the degree of rhizosphere and endophytic colonization from multiwell plate experiments was arcsine square root transformed and analyzed by two-way ANOVA with inoculation method and fungal strain as principal factors, and Bonferroni's multiple comparison post-test. Numerical data from pot experiments was analyzed by ANOVA and Student's t-tests (Motulsky 1995). The analyses were carried out with the statistical software GraphPad Prism 5.00 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Fungal Pathogenicity to CRF

Fungal or (bio)insecticide treatments had a significant effect on the number of surviving larvae in the fungal pathogenicity tests ($F_{10, 153}$ = 8.43; P < 0.0001). T-tests showed a significant increase of mortality caused by *M. brunneum* 1154 and 1868, *B. bassiana*, *T. koningiopsis*, bioinsecticide Naturalis and insecticide Marshal. The two most pathogenic agents were *M. brunneum* 1154 (39.9 ± 9.6% mortality) and *B. bassiana* (38.2 ± 6.9% mortality). The reference biocontrol formulations Naturalis and Delfin and the insecticide Marshal caused a mortality of 38.9 ± 6.6, 23.1 ± 9.7, and 100.0 ± 0.0%, respectively (Fig. 1). Mycelial growth of *M. brunneum* (1154 and 1868) and *B. bassiana* 1174 emerged from eggs and larvae and of Naturalis only from CRF eggs.

Cauliflower–Fungus Interactions in Multiwell Plates

The inoculation method (sporulating agar plugs or seed coating) had a significant effect on plantlet survival in multiwell experiments ($F_{1, 176}$ = 7.35; P = 0.0074). The effect of the individual fungal strain ($F_{7, 176}$ = 1.49; P = 0.174) or the interaction of inoculation method × strain ($F_{1, 176}$ = 0.74; P = 0.637) was not significant. Greatest survival of plantlets was observed in the wells with sporulating agar plugs of *B. bassiana* (100 ± 0%) and the lowest in negative control of 'seed coating' inoculation (surface-sterilized seeds coated with CMC; 30.6 ± 7.2%; Fig. 2).

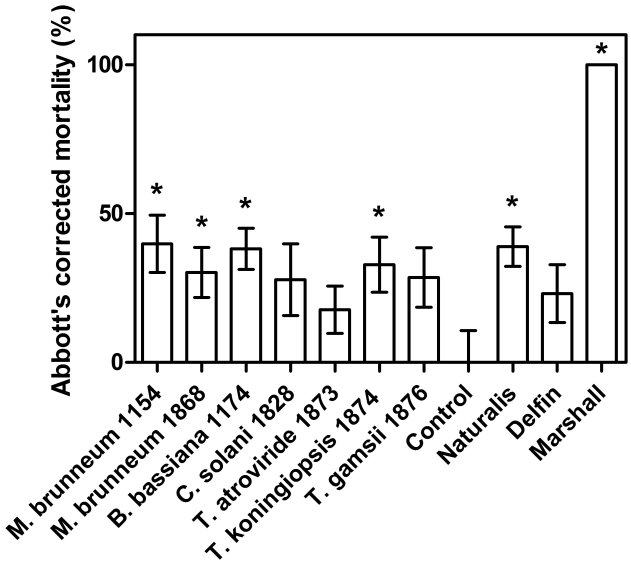

Fig. 1. Mortality of CRF treated with various fungi and three commercial products in *in vitro* bioassays. The experiments were evaluated 14 d after infection to determine the number of surviving larvae. Asterisks denote significant difference from the control (P < 0.05). Data presented are means ± standard error. *Naturalis* – commercial product based on *Beauveria bassiana* ATCC 74040; *Delfin* – commercial product based on *Bacillus thuringiensis* var. *kurstaki*; *Marshal* – commercial insecticide based on carbosulfan.

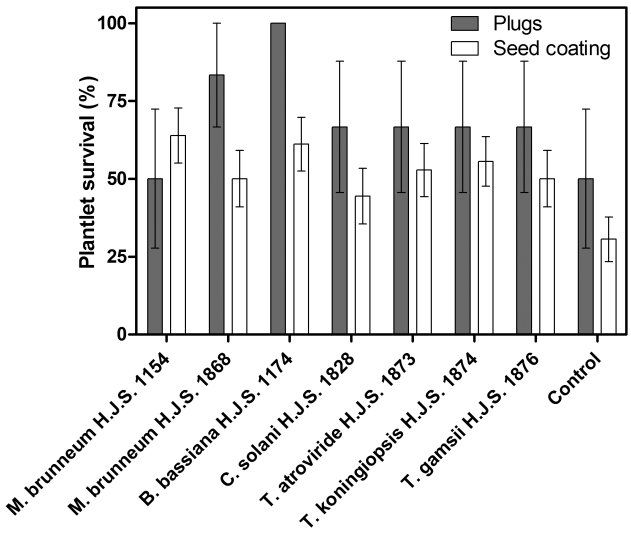

Fig. 2. The fraction of surviving plantlets in multiwell plate experiments. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface-sterilized seeds (Seed coating). Data presented are means ± SE.

The inoculation method ($F_{1, 57}$ = 18.01; P < 0.0001) and fungal strain ($F_{7, 57}$ = 4.72; P = 0.0003) had a significant effect on rhizoplane colonization of the plantlets in multiwell experiments. The interaction of inoculation method × strain ($F_{7, 57}$ = 1.10; P = 0.381) was not significant. Greater rhizoplane colonization was observed when fungi were delivered into test systems as sporulating agar plugs. The roots of plantlets growing above *M. brunneum* 1154 sporulating agar plugs were significantly more colonized by the fungus than roots grown from coated seeds (Fig. 3).

Endophytic tissue colonization was significantly affected by factors inoculation method, fungal taxon, and their interaction in all plant organs investigated (Table 2). The highest and most consistent endophytic colonization was observed in the three *Trichoderma* isolates, which were reisolated from all plant organs investigated. Generally, higher endophytic tissue colonization rate in the three *Trichoderma* spp. isolates was obtained when the plantlets were inoculated via sporulating agar plugs. *M. brunneum* 1154 and

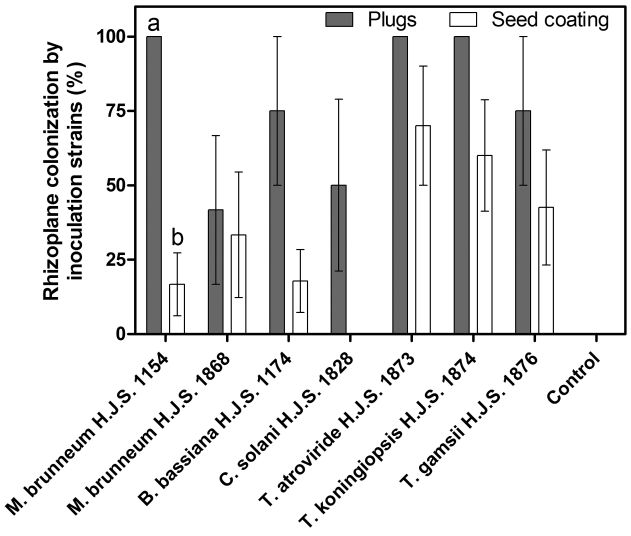

Fig. 3. Rhizoplane colonization of the plantlets in multiwell experiments, expressed as the fraction of infected root pieces. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface-sterilized seeds (Seed coating). Data presented are means ± SE. Bars marked with different lower-case letters are significantly different at P < 0.05.

1868 and *C. solani* 1828 were reisolated sporadically from stems or leaves, but were not found in root tissue. *Beauveria bassiana* 1174 was not reisolated as an endophyte in any plant organ regardless of the inoculation method.

Pot Experiment

The factor fungal strain had a significant effect on plantlet survival in pot experiments ($F_{8, 89}$ = 2.19; P = 0.0356); the effect of inoculation method (drenching or seed coating; $F_{1, 89}$ = 0.08; P = 0.7839) or the interaction of strain × inoculation method ($F_{8, 89}$ = 0.72; P = 0.6806) was not significant. Plantlet survival and fresh biomass increased in pots where *M. brunneum* (isolates 1154 and 1868) conidia were coated onto surface-sterilized seeds compared with plants that emerged from seeds coated without fungus (Fig. 4).

The inoculation method ($F_{1, 87}$ = 12.5; P = 0.0006) had a significant effect on average plantlet weight in pot experiments. The effects of fungal strain ($F_{8, 87}$ = 1.02; P = 0.4298) and the interaction of inoculation method × strain ($F_{8, 87}$ = 0.50; P = 0.8508) had no significant effect on average plantlet weight. Drenching surface-sterilized seeds with *T. atroviride* 1873 resulted in significantly higher average plantlet weight as compared to seed coating application. Coating surface-sterilized seeds with CMC alone significantly reduced average plantlet weight (Fig. 4).

The inoculation method ($F_{1, 88}$ = 4.80; P = 0.0311) had a significant effect on fresh biomass production in pot experiments; the fungal strain ($F_{8, 88}$ = 1.66; P = 0.1188) and the interaction of inoculation method × strain ($F_{8, 88}$ = 0.472; P = 0.8729) was not significant. Surface sterilization and coating seeds with CMC alone significantly reduced fresh biomass production. In contrast, when *M. brunneum* (isolates 1154 and 1868) conidia were coated onto surface-sterilized seeds, fresh biomass production was significantly higher than the respective control, and reached levels statistically indistinguishable to zero control treatment (noncoated, nonsurface-sterilized seeds). Seed surface sterilization did not have a significant effect on plantlet survival, average plantlet weight, or fresh biomass production (Fig. 4).

Discussion

The EPF and other fungal isolates tested in the present study were pathogenic to CRF eggs or larvae, as demonstrated previously

Table 2. Effect of fungal strain and inoculation method on endophytic tissue colonization in different plant organs

Plant organ	Root		Stem		Leaf	
Inoculation method	Plugs	Coating	Plug	Coating	Plug	Coating
M. brunneum 1154	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	11.1 ± 7.0	0.0 ± 0.0	0.0 ± 0.0
M. brunneum 1868	0.0 ± 0.0	0.0 ± 0.0	12.5 ± 12.5	0.0 ± 0.0	12.5 ± 12.5	5.6 ± 5.6
B. bassiana 1174	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C. solani 1828	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	26.7 ± 12.5	0.0 ± 0.0	0.0 ± 0.0
T. atroviride 1873	0.0 ± 0.0	25.0 ± 17.1	100.0 ± 0.0^a	23.6 ± 15.0^b	87.5 ± 12.5^a	27.8 ± 18.1^b
T. koningiopsis 1874	50.0 ± 50.0^a	0.0 ± 0.0^b	50.0 ± 20.4	43.3 ± 19.4	25.0 ± 14.4	30.0 ± 12.2
T. gamsii 1876	83.3 ± 16.7^a	0.0 ± 0.0^b	100.0 ± 0.0^a	25.0 ± 17.1^b	87.5 ± 12.5^a	5.6 ± 5.6^b
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Statistical parameters						
Inoculation method	$F_{1,52}$ = 8.08; P = 0.0064		$F_{1,60}$ = 8.96; P = 0.0040		$F_{1,60}$ = 14.83; P = 0.0003	
Fungal strain	$F_{7,52}$ = 6.43; P < 0.0001		$F_{7,60}$ = 12.31; P < 0.0001		$F_{7,60}$ = 13.17; P < 0.0001	
Interaction	$F_{7,52}$ = 7.11; P < 0.0001		$F_{7,60}$ = 6.43; P < 0.0001		$F_{7,60}$ = 6.76; P < 0.0001	

Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface-sterilized seeds (Coating). Data presented are mean percentages of infected plant tissue pieces ± SE. Values marked with different lower-case letters are significantly different at P < 0.05.

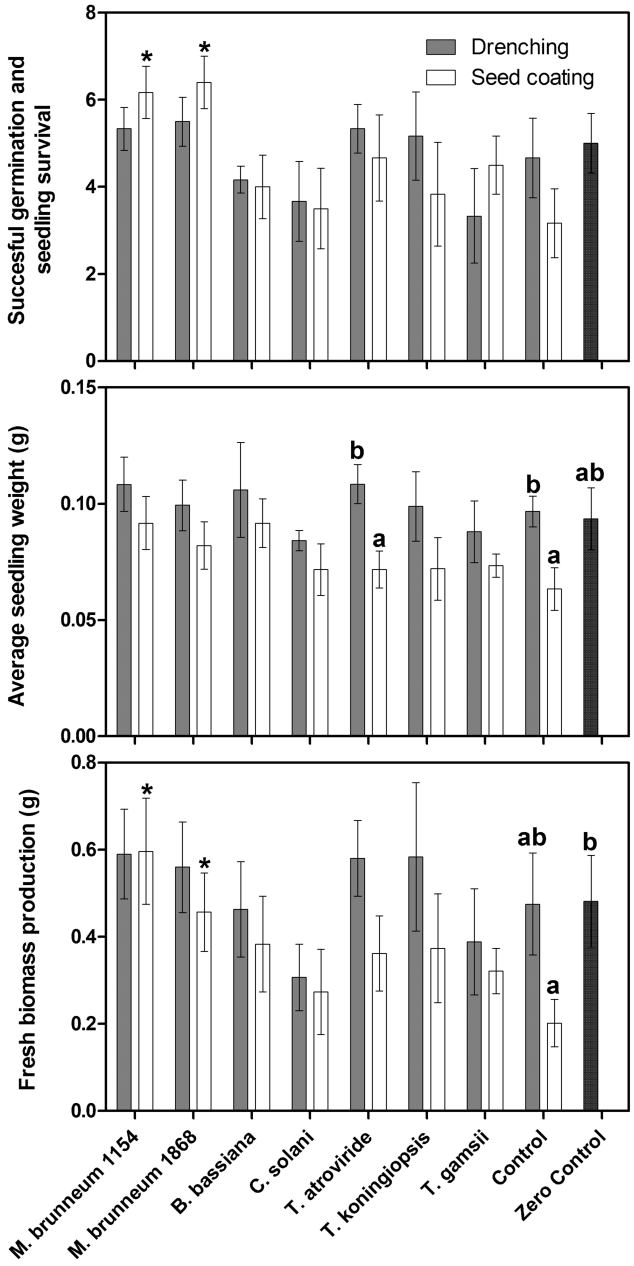

Fig. 4. Effect of fungal strain and inoculation method on plantlet survival, average plantlet weight and fresh biomass production. Inoculation was performed by drenching the growing substratum with conidial suspensions (Drenching) or by coating the conidia onto seeds (Seed coating). Data presented are means ± SE. Bars not sharing the same lower-case letters are significantly different, whereas bars without lower-case letters are statistically indistinguishable. Asterisks (*) denote significant difference from the respective negative controls ($P < 0.05$). Cauliflower seeds used in the Zero control treatment were neither surface sterilized nor drenched, nor coated with conidia.

(Razinger et al. 2014a,b). Additionally, the investigated unformulated fungal taxa could form root associations under controlled laboratory conditions in autoclaved substrate, i.e., they were rhizosphere competent to cauliflower roots as per definitions set by Hu and Leger (2002) and Pava-Ripoll et al. (2011), which may make them suitable biological control agents against soil borne pests, if the virulence of the fungi were not reduced by growing on the plant substrate. Colonizing the rhizosphere, they might have multiple roles in protecting plants from pests and diseases and at the same time promoting plant growth (Vega et al. 2009).

The EPF regularly produced abundant sporulating mycelial outgrowth on CRF eggs and larvae, whereas in the case of *Clonostachys solani* and *Trichoderma* spp., this was not observed. The best performing EPF isolates caused CRF mortality on par with the reference biocontrol agent Naturalis and in range with several other EPF tested against CRF (Vänninen et al. 1999, Chandler and Davidson 2005, Myrand et al. 2015) or the onion maggot *Delia antiqua* (Davidson and Chandler 2005). Additionally, the *M. brunneum* isolates (1154 and 1868) exhibited plant growth promoting effects, highlighting their potential as multifaceted biocontrol agents.

The EPF and *C. solani* were only sporadically reisolated from within plant tissue as endophytes. *Trichoderma* spp. isolates on the other hand were continuously reisolated from within healthy plant tissue and were even able to colonize aboveground plant organs after application by drenching or seed coating. The endophytic trait of the CRF-pathogenic *Trichoderma* spp. isolates tested indicates that these fungi might also confer chewing herbivore resistance to the treated plantlets; however, this mode of action was not evaluated in the present study. On the other hand, endophytism might also cause unwanted food safety-related effects, for example, if the isolates invaded the produce. This would need to be addressed and tested in a potential microbial biopesticide development plan. Although the genera *Beauveria* and *Clonostachys* are not mentioned as common brassica endophytes, *Metarhizium* and *Trichoderma* are (Card et al. 2015). This may indicate that our *Metarhizium* isolates did not possess the traits needed to form an endophytic relationship with cauliflower plantlets.

The evaluation of different fungal application methods revealed that placing sporulating agar plugs below germinating seeds resulted in higher rhizoplane colonization, as well as improved plantlet survival, compared to seed coating procedure. Similarly, a significantly higher endophytic colonization was observed in plantlets growing above *Trichoderma* spp. sporulating agar plugs. Significant effects of different application methods of biologicals on the success of vine weevil biocontrol were published by Ansari and Butt (2012). Successful seed coating application of *M. robertsii* or *M. brunneum* onto wheat seeds, whose roots were later found to be carrying enough conidia to cause significant mortality of *Tenebrio molitor* larvae were reported by Keyser et al. (2014). Likewise, the coating of *M. pingshaense* conidia onto maize kernels resulted in making maize roots pathogenic to the white grub *Anomala cincta* (Peña-Peña et al. 2015). Indeed, if the fungi were applied as a seed treatment and could infect soil pests, such a biological control strategy would be both easier and more economical than inundative delivery of inoculum (Peña-Peña et al. 2015).

A relatively small assortment of fungal isolates exhibited a high variation in rhizosphere competence and endophytism in the laboratory experiments performed with sterilized seeds in sterile substrate. It might be possible that the different tested fungi have a different capacity of cauliflower rhizodeposits utilization and tissue penetration, or that they are differentially susceptible to or able to degrade brassica glucosinolates (Vänninen et al. 1999, Klingen et al. 2002). It would be expected that EPF and other fungi would benefit from rhizosphere exudation, provided they were adapted to soil environments and to the rhizosphere conditions of a specific crop (Pava-Ripoll et al. 2011). Vice versa, the plants would benefit from such a mutualism with rhizosphere-competent insect pathogens (Hu and Leger 2002, Vega et al. 2009, Johnson and Rasmann 2015, Steinwender et al. 2015). We suggest that the first phase of a fungus-based biological control strategy against soil pests should focus on

obtaining isolates well adapted to the crop's specific soil and rhizosphere conditions, potentially even showing plant growth stimulating effects. Next, the isolates would be evaluated for their virulence and pathogenicity to the target pest. This would improve the success probability of such biocontrol attempts, as well as reduce inoculums used and therefore minimize unwanted environmental and nontarget effects.

Conclusions

The study revealed a high variation of rhizosphere competence and endophytic tissue colonization of the tested fungal isolates, as well as significant effects of fungal delivery methods. This indicates several opportunities of future research, namely searching for CRF-pathogenic fungal isolates with a high rhizosphere competence; collaboration with formulation specialists focusing on improving fungal delivery methods which would provide constant colonization pressure and might improve persistence of fungal inoculum on plant roots; cooperation with food and feed safety specialists, checking for potentially harmful effects of the endophytic traits of CRF-pathogenic fungi. In addition, fungi other than solely EPF genera might be considered when investigating such biocontrol strategies.

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